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Replicative senescence in patients with chronic kidney failure

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Replicative senescence in patients with chronic kidney failure.

Background. Chronic activation of immunocompetent cells may lead to stress-induced premature senescence (SIPS); these senescent cells are characterized by a decrease in telomere length. The present study evaluates SIPS in circulating immunocompetent cells from predialysis patients, patients on hemodialysis, and in renal transplant patients with normal renal function.

Methods. Determination of telomere length by flow-fluorescence in situ hybridization (FISH), expression of surface molecules, and evaluation of apoptosis was performed by flow cytometry.

Results. In uremic predialysis patients, we observed a subpopulation of lymphocytes with short telomeres. However, in this population of patients we did not observe SIPS mononuclear cells. In hemodialysis patients, we found a subpopulation of SIPS mononuclear cells that also showed phenotypic changes of proinflammatory activity. Finally, transplant patients with normal renal function also exhibited a subpopulation of SIPS lymphocytes, which can be attributed to chronic lymphocyte activation induced by the major histocompatibility complex.

Conclusion. In chronic kidney disease patients, immunocompetent cells undergo SIPS, a process associated with chronic cell activation and induced by numerous stimuli including uremia, hemodialysis membranes, and bacterial products. Because SIPS immunocompetent cells are activated cells with proinflammatory features and live longer in peripheral blood, it is likely that SIPS cells contribute significantly to the chronic inflammatory state of patients with advanced renal failure.

The inflammatory process is inherent to the immune response induced by injury. When the inflammatory response is excessive or inappropriately controlled, it may result in a chronic systemic inflammatory state with undesired deleterious consequences. Despite advances in end-stage renal disease therapy, it is recognized that 30% to 50% of hemodialysis patients have chronic inflammation. This may be caused by various factors, some of which are related to dialysis and some of which are not. Chronic inflammation in chronic kidney disease (CKD) patients is evidenced by high levels of C-reactive protein and by the presence of proinflammatory cytokines [1–3].

Key words: cellular senescence, chronic kidney disease, inflammation.

It is well established that activation of immunocompetent cells by cytokines or other stimuli may prevent the initiation of the apoptotic program [4, 5]. Thus, activated immunocompetent cells may survive longer in the bloodstream (Fig. 1). The question we addressed in the present study was whether these surviving cells share the characteristics of stress-induced premature senescent cells.

STRESS-INDUCED PREMATURE SENESCENCE

A general characteristic of the aging process is a progressive deterioration of organ function that becomes pathologic with time. It is generally accepted that the decline in cell function with age causes an inability to resist internal or environmental stressors. Thus, internal homeostasis is no longer maintained within physiologic parameters when the aging organism is faced with stress [6, 7].

At the cellular level, the accumulation of injuries causes aging. As a corollary, some evidence of premature cell senescence should be observed if cell damage is artificially increased by sublethal doses of stressing agents. Cell irradiation or oxidative damage results in accelerated cellular senescence, which suggests the presence of stress-induced premature senescence (SIPS) [6, 7]. Cells entering the SIPS process become exhausted and remain in a post-replicative state with a progressive decline in cell function that eventually results in cessation of cell division or growth. These cells may remain in this viable, non-dividing state for months. Theoretically, accumulation of SIPS cells over time may contribute to the pathologic features of aging. For instance, human diploid fibroblasts exposed in vivo and in vitro to proinflammatory cytokines display biomarkers of senescence and might participate in the degradation of the extracellular matrix observed with aging. Furthermore, chronically stressed lymphocytes from acquired immune deficiency syndrome [8] and lupus patients [9] cause immune dysfunction.

Inasmuch as the SIPS process involves changes to almost every aspect of cell function and morphology, cellular senescence is characterized by specific molecular,

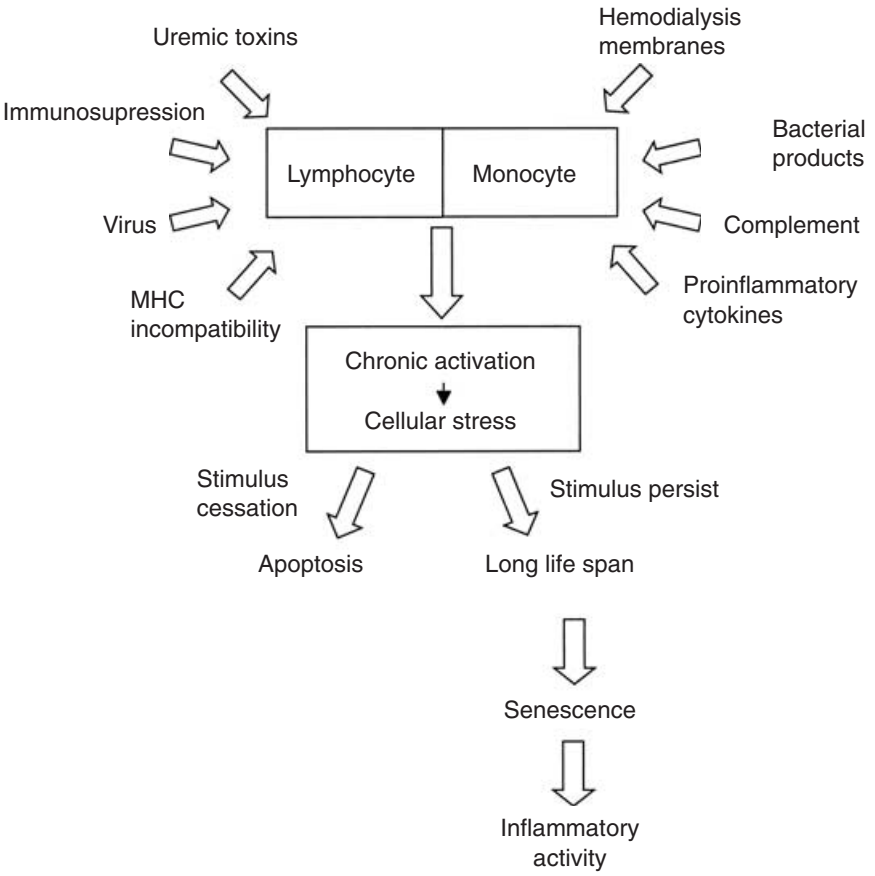

Fig. 1. Factors that are potentially involved in chronic activation and development of SIPS of lymphocyte and monocytes in uremic and hemodialyzed patients.

phenotypic, and functional changes. At the molecular level, one of the markers of senescence is telomere shortening. Accelerated telomere shortening is observed in the majority of cells undergoing SIPS. Human telomeres are simple repeats of the sequence, TTAGGG; in somatic cells, telomeres shorten with each round of replication [10, 11]. It is widely accepted that telomere shortening is a universal mechanism that limits the proliferative potential of normal cells that lack endogenous telomerase. Telomerases are enzymes that replace the telomeric sequence lost with each cell replication. Most human mature cells do not express high levels of telomerase activity and suffer from a progressive erosion of telomeres during each replication. There is a critical level of telomere shortening at which cells enter a post-replicative senescent state.

Stress-induced premature senescent cells also exhibit other functional and morphologic changes: β-galactosidase activity is increased, mitochondrial DNA is deleted, and SIPS mononuclear cells change from CD14bright/CD16dim to CD14dim/CD16bright [10–13].

Previous studies have shown an association between the deterioration of renal function and the degree of kidney senescence as assessed by renal cell telomere shortening [14]. In advanced renal failure, the immune response is altered, which is partly responsible for the increased mortality rate observed among these patients. As a consequence of chronic cell activation, alterations in the immune response may be associated with premature senescence of immunocompetent cells. Therefore, it is reasonable to hypothesize that, in renal failure patients, immunocompromised cells might undergo SIPS. To investigate this hypothesis, we measured telomere length in monocytes and lymphocytes from CKD stage 4, dialysis, and transplant patients.

SIPS IN UREMIC PREDIALYSIS PATIENTS

We have shown that uremia induces lymphocyte cell activation, which is one of the features of the abnormal immune response observed in uremic patients [15]. Extending this line of investigation, we measured telomere length in lymphocytes from uremic patients. The results show the presence of a subpopulation of lymphocytes with short telomeres (Fig. 2). It is likely that a large proportion of SIPS lymphocytes are CD4 cells with Th1 activity, because this type of lymphocyte is activated in uremic patients.

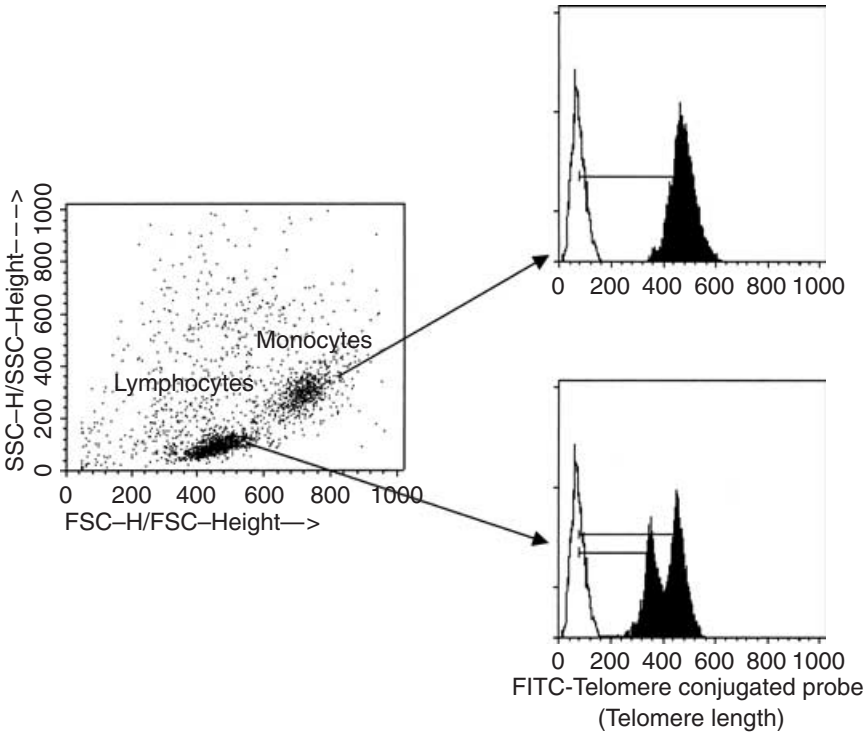

Fig. 2. Representative histogram of telomere length in monocyte cells and lymphocytes from one of the uremic (predialysis) patients evaluated in the present study. There are two populations of lymphocytes showing long and short telomeres, respectively. Mononuclear cells are a single population of cells without short telomeres.

SIPS IN MONONUCLEAR CELLS FROM HEMODIALYSIS PATIENTS

The subpopulation of lymphocytes with short telomeres is absent in patients on regular hemodialysis therapy, which supports the concept that there are other factors involved in lymphocyte SIPS. However, we found that in hemodialysis patients who were dialyzed with cellulosic membranes, a subset of mononuclear cells exhibits characteristics of SIPS such as decreased telomere length and a predominant CD14dim/CD16bright phenotype [16] (Fig. 3). These findings are explained by the fact that uremic patients undergo hemodialysis procedure 3 times weekly, activating mononuclear cells at regular intervals. Consequently, the presence of SIPS mononuclear cells in hemodialysis patients may be the result of increased replicative stress induced by the hemodialysis membrane. Membranes that are less biocompatible induce selective activation of mononuclear cells, which may lead to SIPS. In contrast, exposure of mononuclear cells to membranes that are more biocompatible results in only mild cell activation that does not result in SIPS.

Various authors agree that hemodialysis patients suffer from a chronic inflammatory state [1–3]. This is caused by various stimuli that act simultaneously to activate mononuclear cells, leading to the subsequent inflammatory response. An illustration of the collaborative effect of several stimuli acting on the mononuclear cell of hemodialysis patients is shown in Figure 4. Cytomchrome c oxidase (COX) activation, the most important intracellular pathway mediating proinflammatory activity, was evaluated in normal mononuclear cells incubated with uremic serum and lipopolysaccharide (LPS) in the presence of either cellulosic (Hemophan, Gambro, Germany) or non-cellulosic (AN-69; Hospal, France) hemodialysis membranes. Compared with AN-69, the hemophan membrane produced an increase in the production of COX messenger RNA, COX protein, and prostaglandin E (PGE).

The percentage of SIPS mononuclear cells in hemodialysis patients treated with cellulosic membranes correlated significantly with serum levels of C-reactive protein, a parameter that reflects inflammatory activity (data not shown). This finding supports the theory that, as observed in other patients with chronic inflammatory diseases, hemodialysis patients maintain a large percentage of senescent mononuclear cells in peripheral blood, which is associated with the chronic inflammatory state.

Moreover, we have observed that SIPS mononuclear cells from our hemodialysis patients exhibit increased levels of intracytoplasmic proinflammatory cytokines, suggesting that SIPS cells are preactivated mononuclear cells. In vitro, these cells release large amounts of proinflammatory cytokines in response to substimulatory doses of LPS or bacterial DNA. These results suggest that these cells contribute to a chronic inflammatory state. Nevertheless, according to our data, SIPS mononuclear cells in hemodialysis patients are both the cause and the consequence of chronic inflammation.

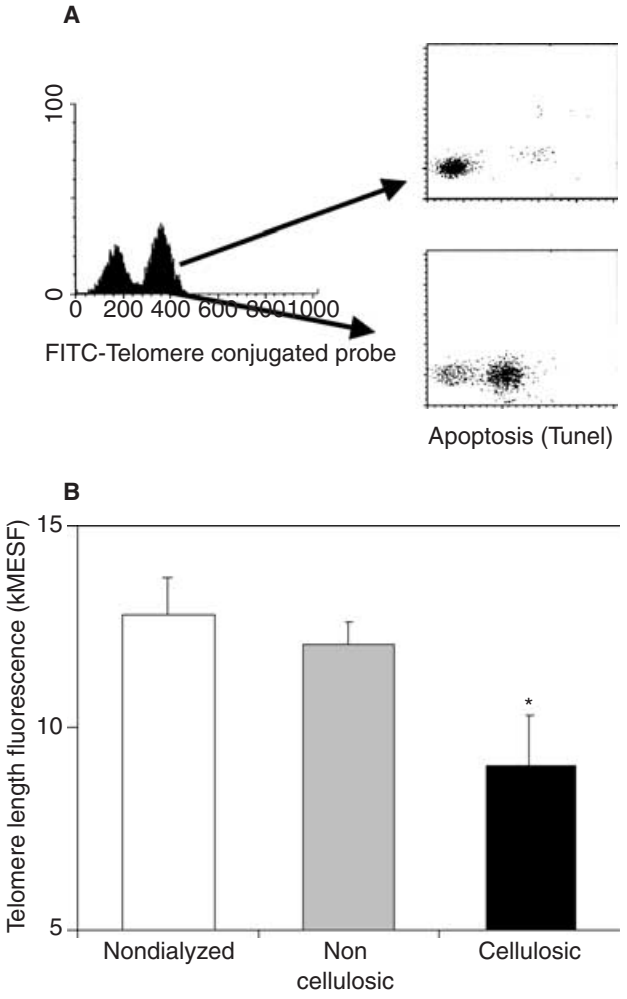

Fig. 3. (*A*) Representative studies showing telomere length and apoptosis in a sample of mononuclear cells from a hemodialysis patient included in the study. Mononuclear cells with long telomeres show a small percentage of apoptosis, whereas most of the mononuclear cells with short telomeres undergo apoptosis in vitro. (*B*) Mean telomere length of mononuclear cells from predialysis patients and hemodialysis patients treated with cellulosic or noncellulosic hemodialysis membranes. Mean telomere length is decreased in mononuclear cells from hemodialysis patients treated with cellolosic membranes as compared with noncellulosic and predialysis patients.

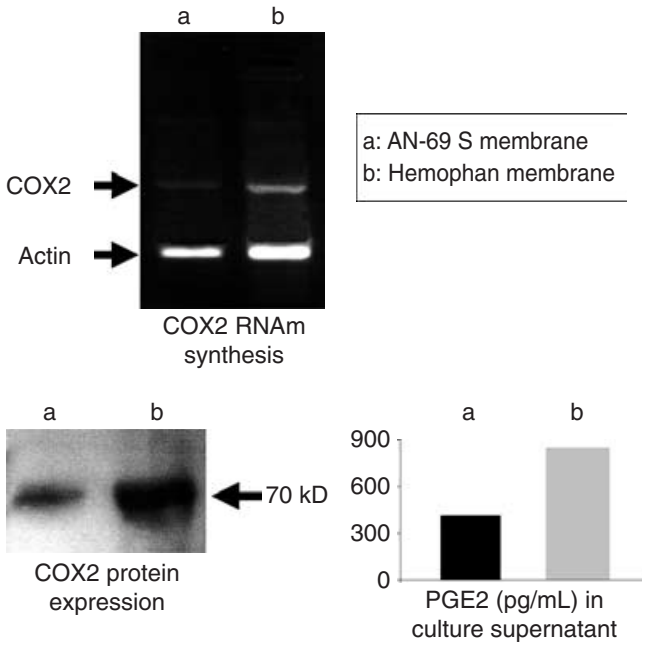

Fig. 4. Mononuclear cells from a healthy patient cultured with uremic serum and LPS in the presence of a cellulosic membrane (hemophan) exhibit a high degree of proinflammatory activity as illustrated by the high expression of COX messenger RNA and protein, and the increased production of PGE2. However, when these cells were cultured under the same conditions, but in the presence of noncellulosic membranes (AN-69), only a low degree of inflammatory response is observed.

SIPS IN RENAL TRANSPLANTS

A subpopulation of lymphocytes (17% ± 8%) with short telomeres is found in renal transplant patients even when renal function is close to normal (a serum creatinine concentration less than 2 mg/dL). Analysis of telomere length of mononuclear cells revealed no significant difference between those of renal transplant patients and healthy individuals. Other authors have reported similar observations [17]. Although uremia was no longer present in these patients, there are other factors such as the immune response to the graft and the immunosuppression therapy, which may explain the activation of lymphocytes and subsequent development of SIPS. It remains to be clarified whether SIPS is restricted to lymphocytes showing evidence of chronic activation, and whether the presence of SIPS lymphocytes in transplant recipients has clinical significance.

CONCLUSION

In CKD patients, immunocompetent cells undergo SIPS, a process associated with chronic cell activation. Uremia, hemodialysis therapy, and infections cause immunocompetent cell activation and premature senescence. Each type of immunocompetent cell reacts differently to the various stimuli present in the uremic patient. In predialysis patients, SIPS is observed in lymphocytes; in contrast, in hemodialysis patients, mononuclear cells exposed to the hemodialysis membrane are chronically activated and develop SIPS. In transplant patients, we observed a subpopulation of SIPS lymphocytes that may be the result of major histocompatibility complex (MHC) incompatibility and immunosuppressive therapy. As SIPS immunocompetent cells are activated cells with proinflammatory activity and live longer in peripheral blood, it is our belief that the accumulation of these cells contributes substantially to the chronic inflammatory state of CKD patients.

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